



FIGURE 1

CHOP

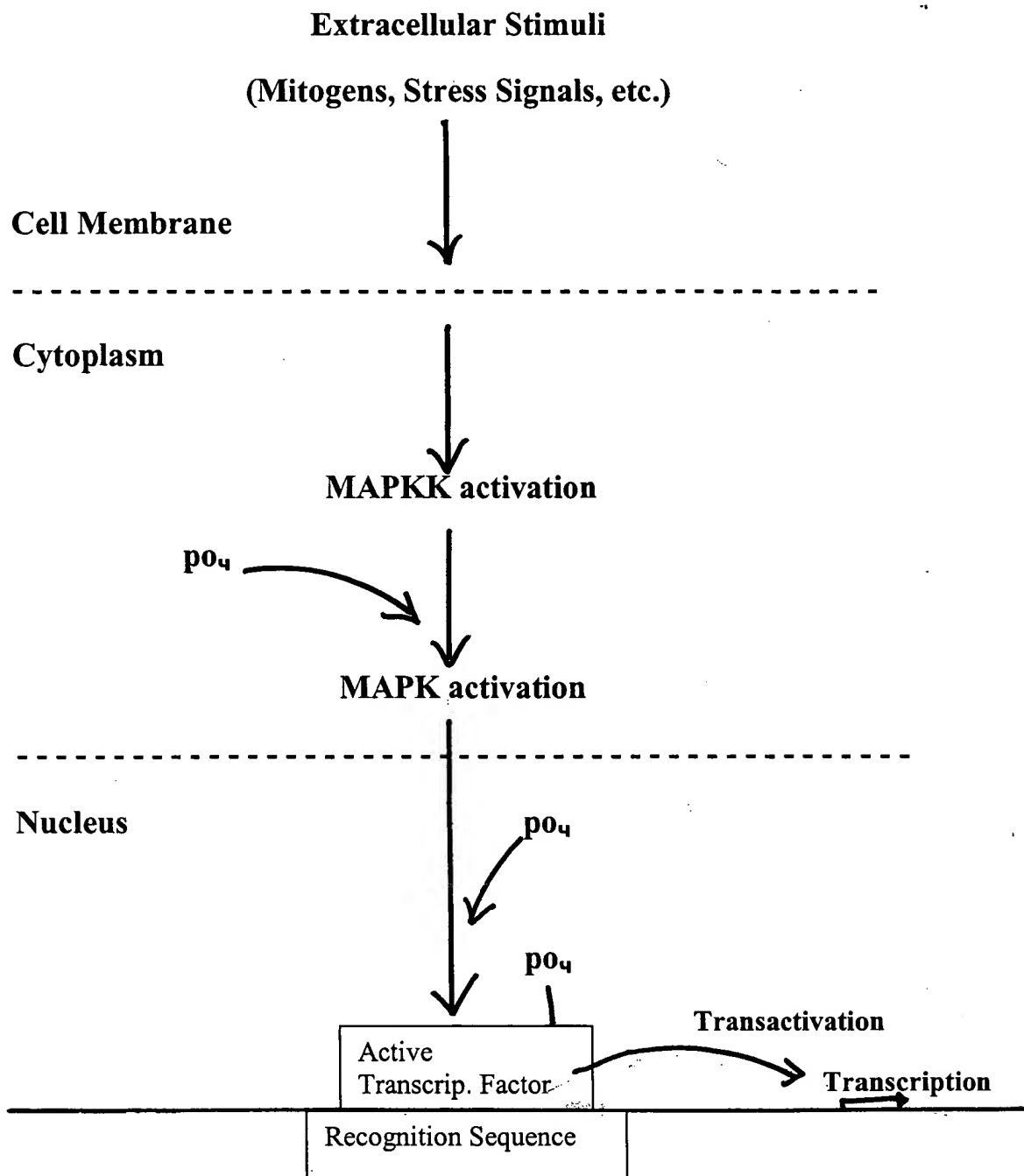


FIGURE 2

Monitoring Pathway-Specific Signal Transduction

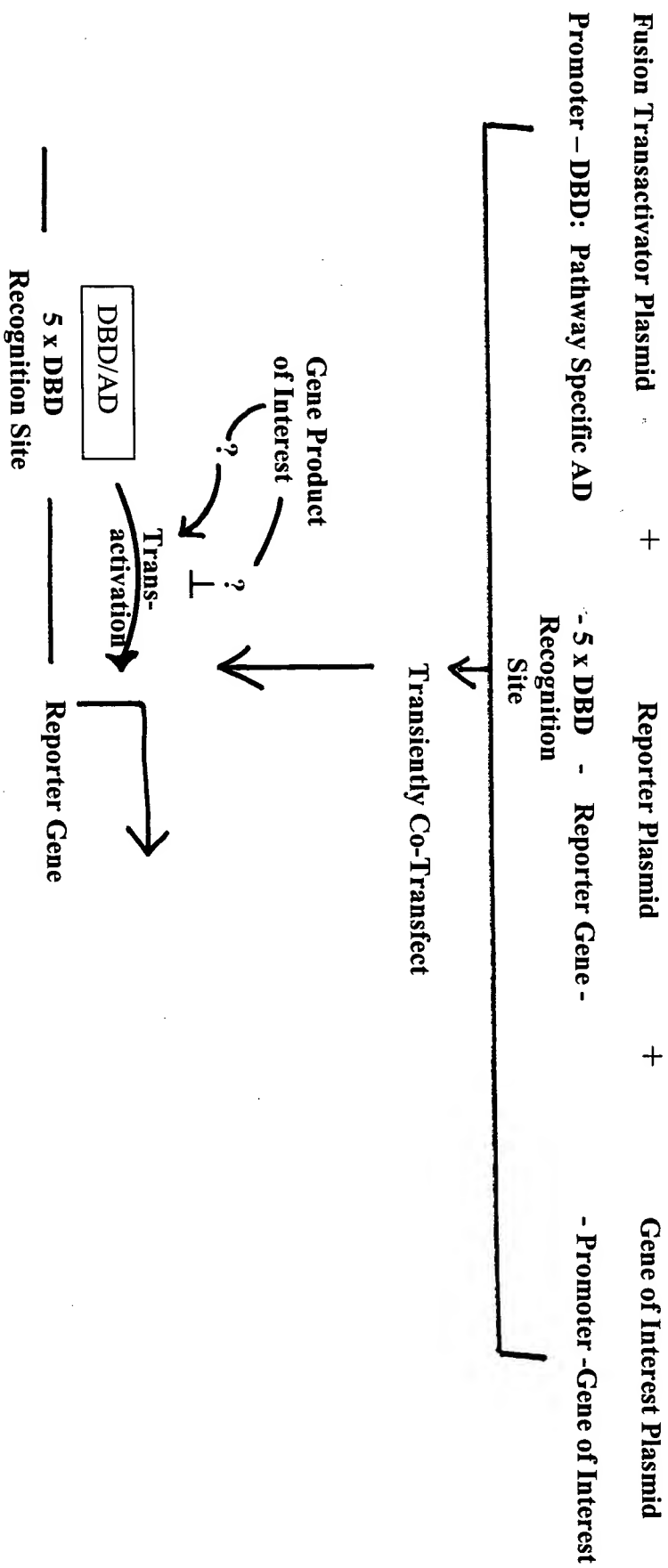




FIGURE 3

Selection of Stable Reporter Cell Lines

Reporter Construct (w/linked DBD element)



Stably transfect



Screen for clones with low background
of Reporter activity and strong
response to DBD – bearing activator(s)



“Stable Reporter Cell Line”



Stably transfect with fusion
transactivator plasmid



Screen for clones with strong
response to pathway-specific
upstream activator(s)

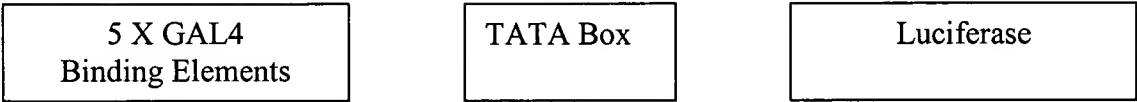


“Pathway-Specific Stable Reporter Cell Line”



FIGURE 4

4.1.1. pFR-Luc Plasmid



Sequence of GAL4 Binding Element in the pFR-Luc Plasmid

GT CGGACTACTGTCCTCCG AG CGGAGTACTGTCCTCCG SEQ ID NO:9
AG CGGAGTACTGTCCTCCG AG CGGAGTACTGTCCTCCG SEQ ID NO: 10
AG CGGAGTACTGTCCTCCG AG CGGAGACTCTAGAGGG SEQ ID NO: 11
TATATAATGGATCCCCGGGT AC CGAGCTCGAATTC - - SEQ ID NO: 5
--CAGCTTGGCATTCCGGTACTGTTGGTAAATG--Luciferase SEQ ID NO: 6



FIGURE 5

4.1.2. Fusion Transactivator Plasmids

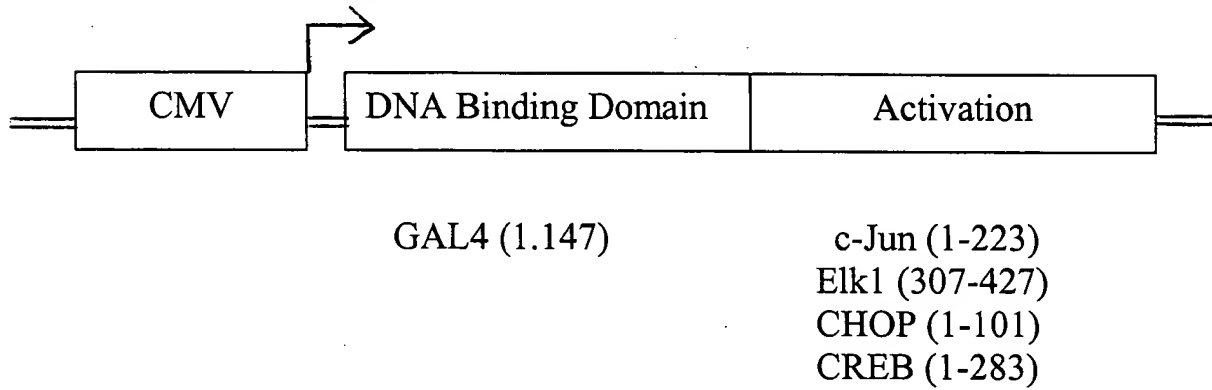
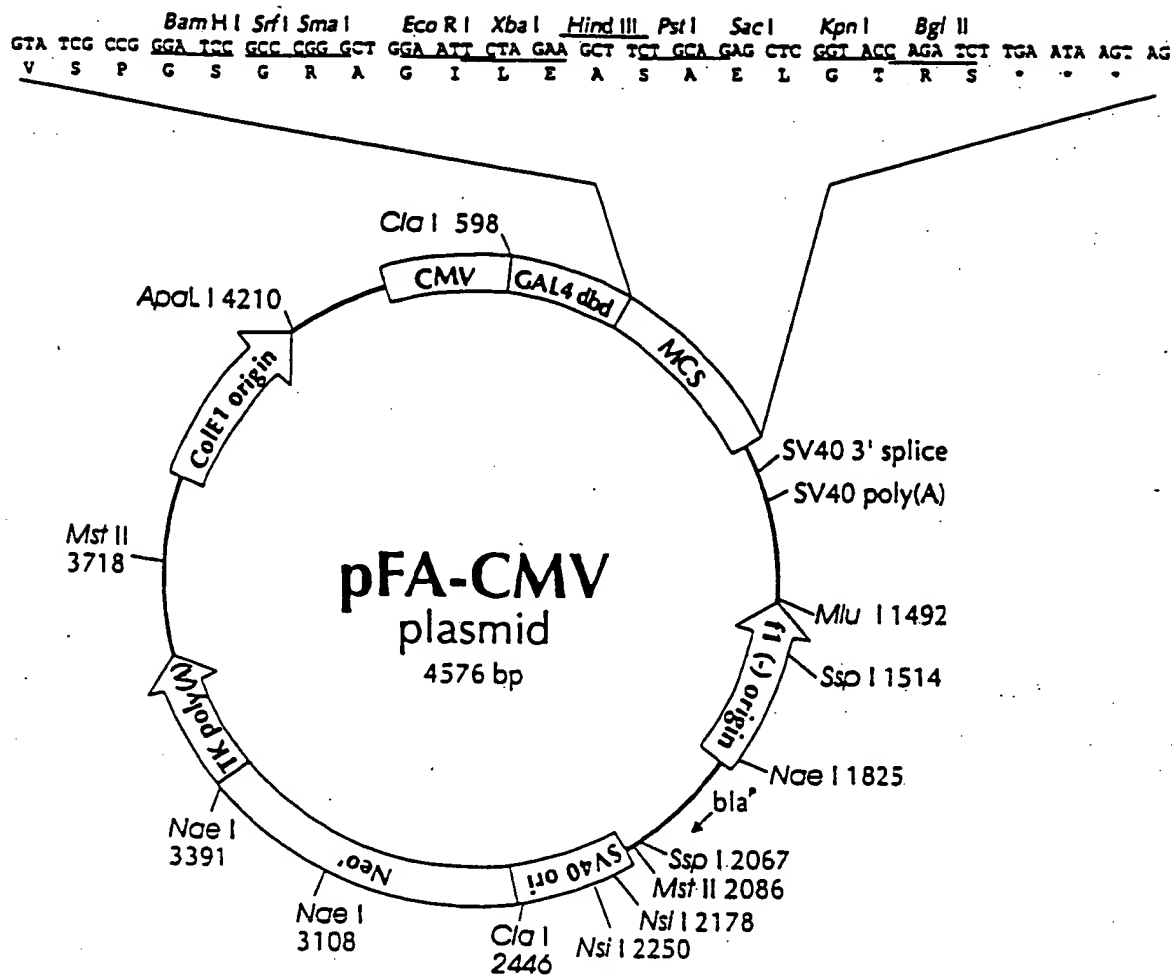




FIGURE 6

4.1.4. pFA-CMV Plasmid



4.2 Preparation of medium and reagents

- Luciferase Assay Reagent (1 x)**
- 40.0mM tris(hydroxymethyl)aminomethane (pH7.8)
 - 0.5 mM ATP
 - 10 mM MgSO₄
 - 0.5 mM EDTA
 - 10.0 mM DDT
 - 0.5 mM coenzyme A
 - 0.5 mM Luciferin

- Cell Lysis Buffer (5 x)**
- 40 mM tris(hydroxymethyl)aminomethane (pH 7.8)
 - 50 mM NaCl
 - 2 mM EDTA
 - 1 mM MgSO₄
 - 5 mM DTT
 - 1% Triton® X-100



FIGURE 7

4.1.3. Control Plasmids

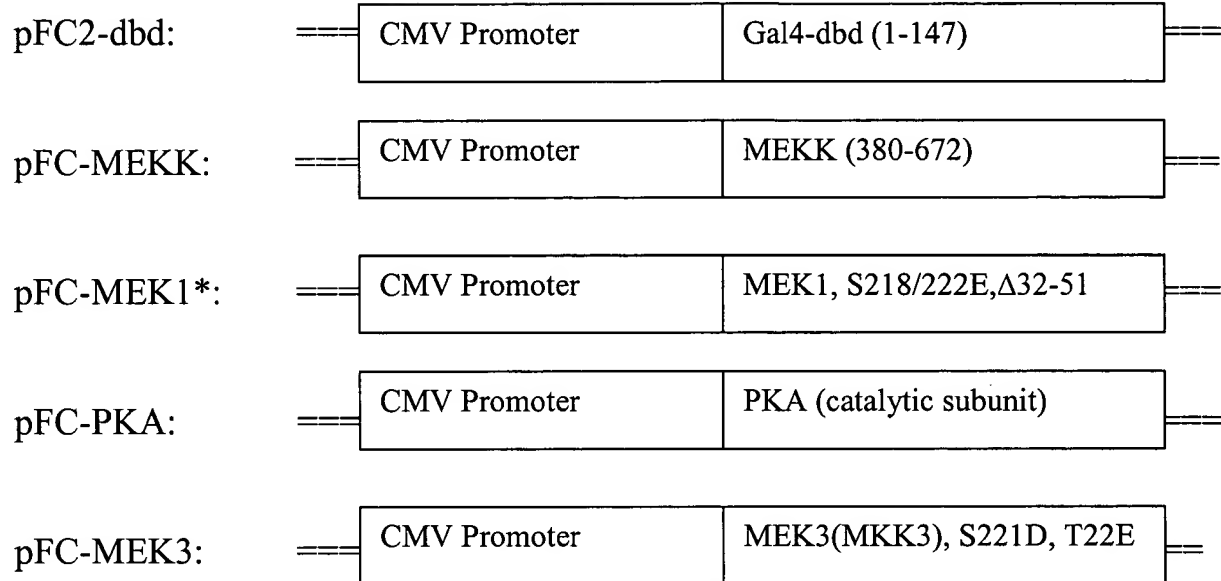




FIGURE 8

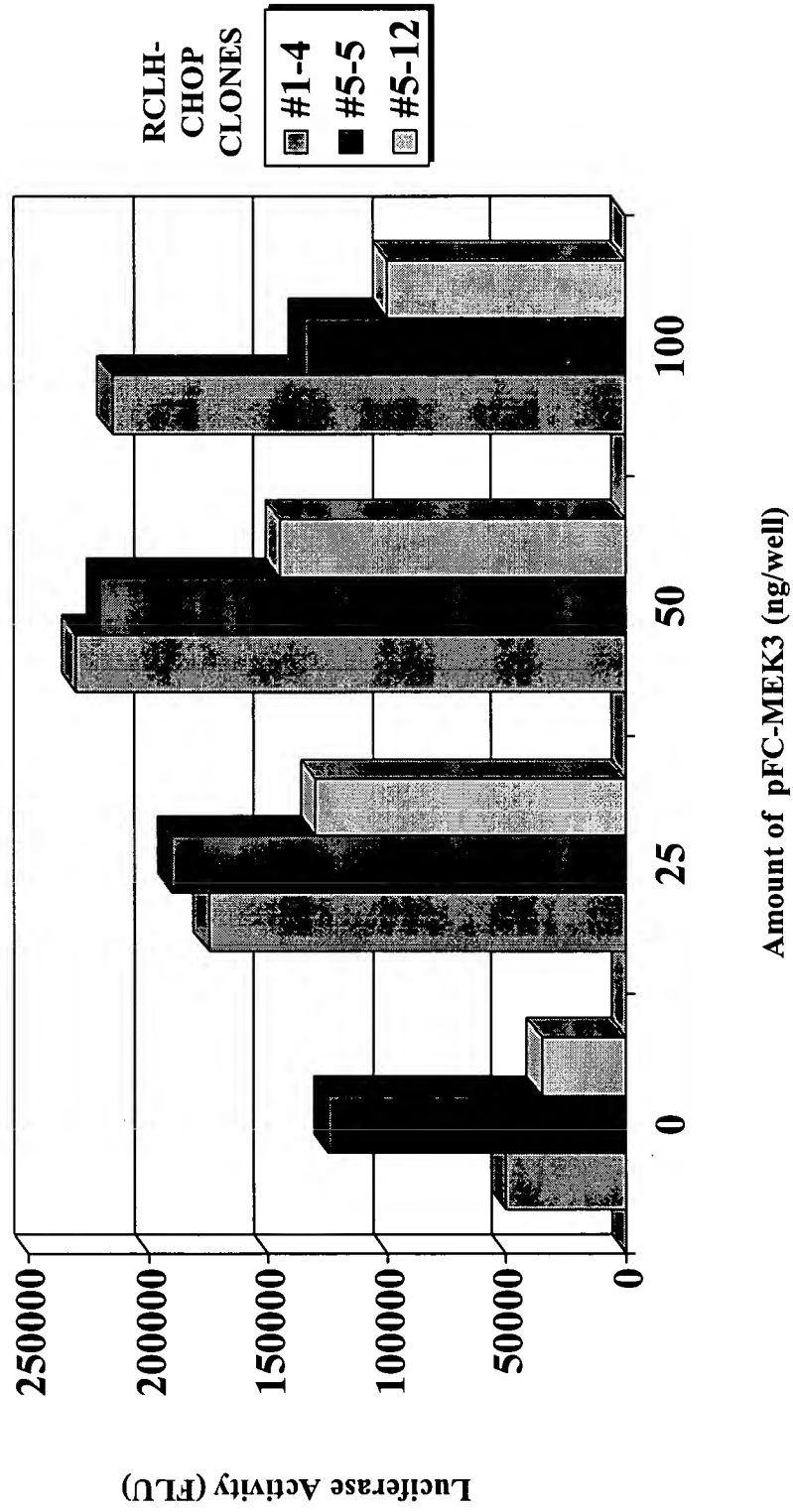




FIGURE 9

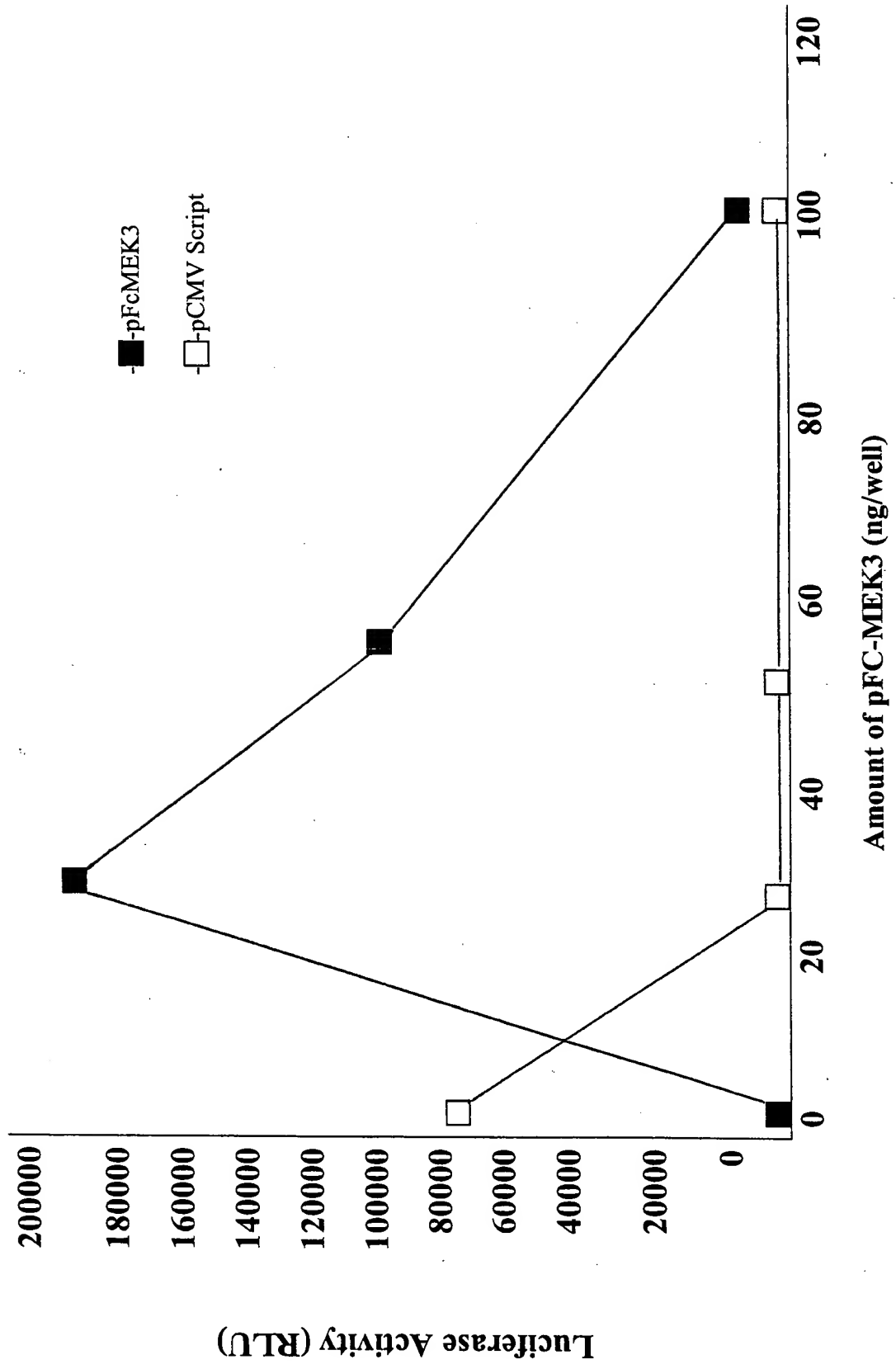


FIGURE 10

